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**Evaluation of regional and distant metastases in head and neck squamous
cell carcinoma patients with special reference to the assessment of regional
lymph nodes in oral cancer**

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Academic dissertation

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Abstract

Mucosal squamous cell carcinoma of the head and neck (HNSCC) often metastasises regionally via lymphatic routes in the early course of the disease, while distant spread is usually encountered in patients with advanced disease. For optimal treatment planning, a thorough assessment of the metastatic status of the tumour is required. Current imaging methods do not allow the recognition of all patients with metastatic disease. Therefore, elective treatment of the cervical lymph nodes is usually given to patients in whom the risk of subclinical metastasis is estimated to exceed 15-20%. The objective of this study was to improve the pre-treatment evaluation of patients diagnosed with HNSCC. Particularly, we aimed at improving the recognition of patients who will benefit from elective neck treatment.

Computed tomography (CT) of the chest and abdomen was performed prospectively for 100 previously untreated patients diagnosed with HNSCC and the findings were analysed to clarify the indications for this examination in this patient group. CT of the chest influenced the treatment approach in 3% of patients, while CT of the abdomen did not reveal any significant findings. Our results suggest that CT of the chest and abdomen is not indicated routinely for patients with newly diagnosed HNSCC but can be considered in selected cases.

Retrospective analysis of 80 patients treated for early stage squamous cell carcinoma of the oral tongue was performed to investigate the potential benefits of elective neck treatment and to examine whether histopathological features of the primary tumour could be used in the prediction of occult metastases, local recurrence, or/and poor survival. Patients who had received elective neck treatment had fewer cervical recurrences during the follow-up when compared to those who only had close observation of the cervical lymph nodes. The difference was statistically significant. Elective neck treatment did not result in survival benefit, however. Of the histopathological parameters examined, depth of infiltration and pT-category (representing tumour diameter) predicted occult cervical metastasis, but only the pT-category predicted local recurrence. Depth of infiltration can be used in the identification of at risk patients but no clear cut-off value separating high-risk and low-risk patients was found. None of the histopathological parameters examined predicted survival.

Sentinel lymph node (SLN) biopsy was studied as a means of diagnosing patients with subclinical cervical metastases. Sentinel lymph node biopsy was applied to 46 patients who underwent elective neck dissection for oral squamous cell carcinoma. In addition, SLN biopsy was applied to 13 patients with small oral cavity tumours who were not intended to undergo elective neck dissection because of low risk of occult metastasis. The sensitivity of SLN biopsy for finding subclinical cervical metastases was found to be 67%, when SLN status was compared to the metastatic status of the rest of the neck dissection specimen. Of the patients not planned to have elective neck dissection, SLN biopsy revealed cervical metastasis in 15% of the patients. Our results suggest that SLN biopsy cannot yet entirely replace elective neck dissection in the treatment of oral cancer, but it seems beneficial for patients with low risk of

metastasis who are not intended for elective neck treatment according to current treatment protocols.

List of original publications

This study is based on the following publications referred to in the text by their roman numerals.

- I** Keski-Säntti H, Markkola A, Mäkitie AA, Bäck L, Atula T. CT of chest and abdomen in patients with newly diagnosed head and neck squamous cell carcinoma. *Head Neck* 27:909-15, 2005.
- II** Keski-Säntti H, Atula T, Törnwall J, Koivunen P, Mäkitie A. Elective neck treatment versus observation in patients with T1/T2 N0 squamous cell carcinoma of oral tongue. *Oral Oncol* 42:96-101, 2006.
- III** Keski-Säntti H, Atula T, Tikka J, Hollmén J, Mäkitie AA, Leivo I. Predictive value of histopathologic parameters in early squamous cell carcinoma of oral tongue. *Oral Oncol*. In press. Available online 15 February 2007.
- IV** Keski-Säntti H, Kontio R, Törnwall J, Leivo I, Mätzke S, Suominen S, Leppänen E, Atula T. Sentinel lymph node biopsy or elective neck dissection for patients with oral squamous cell carcinoma? *Eur Arch Otorhinolaryngol*. In press. Available online 2 October 2007.
- V** Keski-Säntti H, Kontio R, Leivo I, Törnwall J, Mätzke S, Mäkitie AA, Atula T. Sentinel lymph node biopsy as an alternative to wait and see policy in patients with small T1 oral cavity squamous cell carcinoma. *Acta Oto-Laryngol*. In Press. Available online 11 June 2007.

Abbreviations

CT	Computed tomography
DM	Distant metastasis
DSS	Disease specific survival
ELNT group	Elective neck treatment group (Study II)
END	Elective neck dissection
H&E	Hematoxylin and eosin
HNSCC	Head and neck squamous cell carcinoma
MRI	Magnetic resonance imaging
OBS group	Observation group (Study II)
OS	Overall survival
PET	Positron emission tomography
RT	Radiotherapy
SCC	Squamous cell carcinoma
SLN	Sentinel lymph node
US	Ultrasound

1. Introduction

Globally, about 650 000 new cancers of oral cavity, pharynx and larynx are detected annually and they account for about 350 000 annual deaths (Parkin et al., 2005). In Europe, great variation exists in the incidence rates of head and neck cancer among male population. In France, the world standardised incidence rate of oral cavity, pharynx and larynx cancer in men is approximately 50/100000, while in Finland it is about 10/100000 (Black et al., 1997). Among females, the risk of head and neck cancer is substantially lower and there is much less variation in the incidence rates. Higher incidence rates, i.e. 150/100000, have been reported in Asia (Siddiquee et al., 2006). In Finland, according to the Finnish Cancer Registry, about 400 new cancers of the oral cavity, pharynx and larynx are encountered annually (Cancer Statistics). Of all the malignant tumours in these head and neck sites, squamous cell carcinoma (SCC) covers over 90% (Shah and Patel, 2003). The main risk factors for head and neck squamous cell carcinoma (HNSCC) are tobacco and alcohol consumption and the combined use of these products results in multiplication of the risk (Blot et al., 1988; Macfarlane et al., 1995). Traditionally, the treatment of HNSCC is based on surgery, radiotherapy (RT), or a combination of these treatment modalities. In recent years, chemotherapy in combination with RT (concomitant chemoradiation) has increasingly replaced surgery as primary treatment for advanced tumours, while surgery is more often preserved for salvage treatment (Forastiere A, 2001; Brockstein and Vokes, 2004; American Society of Clinical Oncology, 2006). Primary tumour site and neck are the most frequent sites for treatment failures and the cure rates of locoregionally recurrent disease are generally reported to be poor (Kowalski, 2002; Wong et al., 2003). Therefore, primary treatment should be efficient enough to achieve a permanent control of the disease.

Accurate pre-treatment evaluation of patients with HNSCC is fundamental for optimal treatment planning. A thorough assessment of the primary tumour and the metastatic status of the disease is required. Particularly important is the evaluation of the status of the regional (cervical) lymph nodes, which is the most important prognostic factor in patients diagnosed with HNSCC (Shah and Patel, 2003). When regionally metastatic disease is detected, it is beyond doubt that the neck has to be treated, provided that systemic metastases are not present. Patients with no clinical or radiological signs of metastatic disease present a clinical problem. The incidence of subclinical cervical metastases has been reported to be high even in patients with T1- and T2-tumours (Byers et al., 1998; Esposito et al., 2001; Haddadin et al., 1999; Po Wing Yuen et al., 2002). The imaging methods currently in use do not allow the recognition of all patients with metastatic disease (Castelijns and van den Brekel, 2002; Stuckensen et al., 2000). Therefore, a common practice is to treat the neck electively, when the risk of occult cervical metastases is estimated to be over 15-20% (van den Brekel et al., 1993; Wei et al., 2006). This kind of treatment regimen leads to over-treatment of patients with no metastatic disease and on the other hand, cervical metastases of some patients are initially left untreated. Therefore, better methods for finding patients with occult neck disease are needed.

When distant metastasis (DM) is present at the time of diagnosis, curative treatment is usually not possible. If the patient has a second primary tumour, it may be possible to treat both tumours with curative intent (Kuriakose et al., 2002; Hujala, Sipilä and Grénman, 2005). Computed tomography (CT) of the chest is used in many centers to rule out DM and second primary tumours. The indications for chest CT in patients with HNSCC are not clear, however. Contradictory results about its benefits in this patient group have been published (Mercader et al., 1997; Nilssen et al., 1999; Reiner et al., 1997; Tan et al., 1999).

The aim of this work was to improve the pre-treatment evaluation of patients diagnosed with HNSCC. The benefits of routine screening for DM and pulmonary and oesophageal second primary tumours with CT of the chest and abdomen were studied to clarify the indications for this examination. Of particular interest was to improve the recognition of patients who will benefit from elective neck treatment. The benefits of elective neck treatment and the ability of histopathological features of the primary tumour to predict occult metastases and/or poor outcome were examined in patients with early stage SCC of the oral tongue. In addition, sentinel lymph node (SLN) biopsy was studied as a means of diagnosing patients with subclinical cervical metastases.

2. Review of the literature

2.1. Head and neck squamous cell carcinoma

2.1.1. General considerations

The 5-year survival rate for patients diagnosed with oral cavity or pharyngeal cancer in the United States between 1992 and 1997 is 56.3%; while for patients diagnosed with laryngeal cancer the 5-year survival rate is 63.5% (Carvalho et al., 2005). Comparable survival rates have been reported for Finnish patients diagnosed with cancer at these sites (Dickman et al., 1999). These figures do not give a precise description of the survival rates for these malignancies, as great variation in survival exists between different patient groups depending on the site of the tumour and the stage of the disease.

The main prognostic indicator of HNSCC is the TNM stage at diagnosis. An inverse correlation exists between increasing stage and survival, e.g. the 5-year disease specific survival (DSS) has been reported to range from 91% for Stage I disease to 3.6% for Stage IVC disease in all patients with mucosal head and neck cancer (Iro and Waldfahrer, 1998). The DSS of patients with oral tongue SCC of various stages in Finland is presented in Figure 1 (Mäkitie et al., 2007). The presence or absence of cervical lymph node metastasis has long been recognised as the most important prognostic factor in patients with HNSCC who do not have DM. With regional metastasis present at the time of diagnosis, the cure rates drop roughly to half (Alvi and Johnson, 1996; Baatenburg de Jong et al., 2001; Teichgraeber and Clairmont, 1984). Patients diagnosed with DM are generally considered incurable and only palliative treatment is offered. Practically all these patients die of their disease, usually within one year (Calhoun et al., 1994).

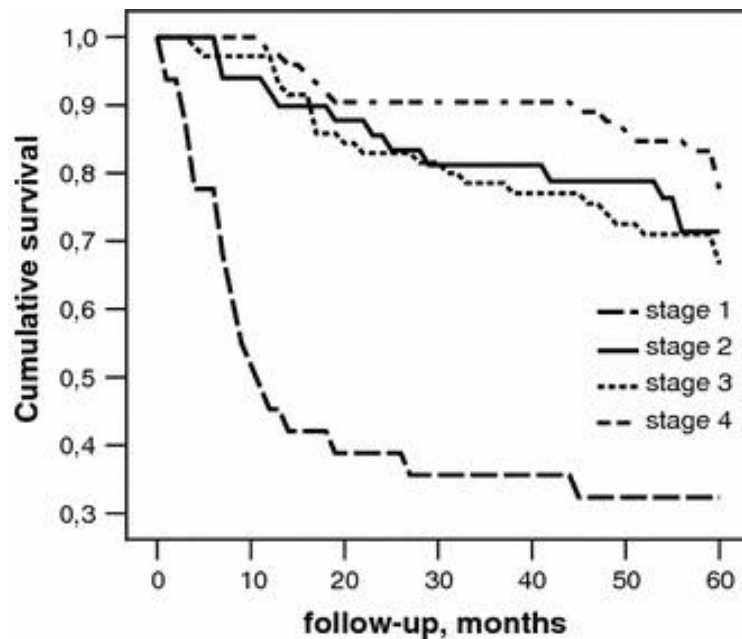


Figure 1. Disease specific survival of patients with oral tongue carcinoma in Finland in 1995-1999 (Mäkitie et al., 2007).

Prognosis of the patients also depends on the site of the primary tumour (Baatenburg de Jong et al., 2001; Berrino and Gatta, 1998). In European patients diagnosed with HNSCC between 1985 and 1989, the tumour site with the most unfavourable prognosis is hypopharynx and with the most favourable prognosis is larynx; the 5-year relative survival rates for tumours of these sites are 22% and 63%, respectively (Berrino and Gatta, 1998). In that study remarkable differences in survival were found even between different subsites within one tumour site. The relative risk associated with supraglottic laryngeal cancer was 2.2 fold the risk associated with glottic cancer. In the oropharynx, the risk associated with tonsillar cancer was substantially lower than that of other oropharyngeal subsites. The differences in survival according to tumour location can in part be explained by different stage distribution at diagnosis and different possibilities of treatment.

The cornerstones in the treatment of HNSCC are surgery and radiotherapy. For early lesions, either of these modalities can be used alone. For advanced lesions, combined therapy (surgery plus RT) is advisable. A change in the treatment protocols has taken place in recent years, however. Concomitant chemoradiotherapy is increasingly used as primary treatment for advanced tumours instead of surgery as it allows organ preservation, while surgery is preserved for salvage treatment (Forastiere et al., 2001; American Society of Clinical Oncology, 2006; Brockstein and Vokes, 2004). It seems that because of organ preservation, patients may achieve better long-term quality of life after chemoradiotherapy compared with the use of conventional surgery and postoperative radiation (Nguyen et al., 2002). Chemoradiotherapy is currently recommended as an adjuvant therapy in the postoperative setting for patients with high risk for recurrence instead of RT alone (Brockstein and Vokes, 2004). The preferred treatment depends not only on the site and stage of the tumour, but also

on the expertise of the institute where the treatment is given. In addition, many patient-related aspects need to be considered when choosing the most appropriate treatment for an individual patient.

Overall, despite the progresses in treatment, only modest improvement in the prognosis of patients diagnosed with HNSCC has been reported in the last decades. In Finland and in the United States, the most favourable improvement in survival rates has been reported in the treatment of pharynx cancer. The prognosis of larynx cancer patients has improved only very slightly in Finland and even decreased in the United States. The prognosis of oral cavity cancer has remained about the same (Dickman et al., 1999; Jemal et al., 2002; Carvalho et al., 2005).

2.1.2. Metastatic patterns

In the western countries, 40-50% of all patients diagnosed with HNSCC present with metastatic disease (Lindberg, 1972; Iro and Waldfahrer, 1998; Dickman et al., 1999; Shah and Patel, 2003; Carvalho et al., 2004). The probability of having metastatic disease at presentation highly depends on the site of the primary tumour. Patients with lip cancer usually present with only local disease, while the majority of pharynx cancer patients present with advanced stage disease with regional and/or distant metastasis (Dickman et al., 1999; Carvalho et al., 2004). The risk factors for having regional or distant metastases will be discussed later in chapter 2.3. Spread to cervical lymph nodes can occur in the early course of HNSCC (Byers et al., 1998; Esposito et al., 2001; Haddadin et al., 1999; Hicks et al., 1999; Po Wing Yuen et al., 2002). For the purpose of systematic description of clinical and pathological findings, the cervical lymph nodes have been divided into different levels (Figure 2.) (Robbins et al., 1991; Robbins et al., 2002). The pattern of lymphogenic spread to different neck levels depends on the site of the primary tumour (Lindberg, 1972; Shah, 1990). In oral cavity tumours, metastatic lymph nodes are most often present in levels I-III. In oropharyngeal, hypopharyngeal, and laryngeal tumours, the most often affected levels are II-IV. From the tumour sites mentioned above, metastatic spread to level V is rare even in patients with metastatic lymph nodes in other neck levels (Candela, Kothari and Shah, 1990; Shah, 1990). From the nasopharyngeal tumours, metastatic spread occurs predominantly to levels II-III and V. Bilateral metastases are frequently encountered in tumours of the base of tongue, nasopharynx and supraglottic larynx (Lindberg, 1972).

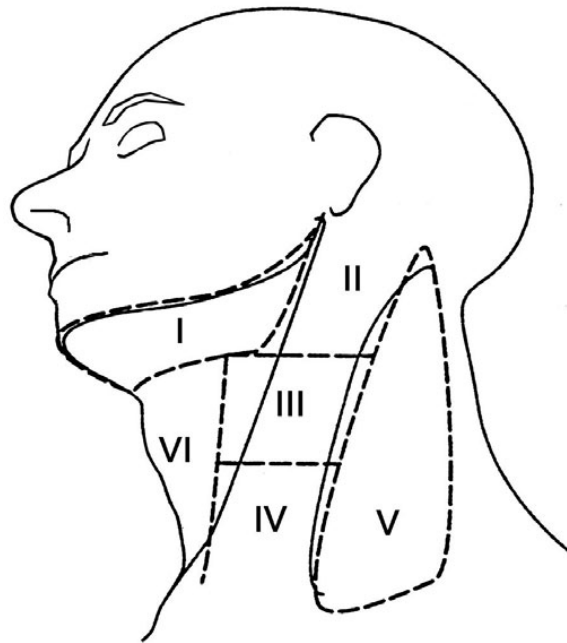


Figure 2. Cervical levels of lymphatic distribution

Distant metastatic spread occurs relatively late in the course of the disease and it is thought to be haematogenic (Calhoun et al., 1994; Leemans et al., 1993; Leon et al., 2000). Surprisingly, there are not many studies reporting on the incidence of DM at presentation in patients diagnosed with HNSCC. According to Dennington and colleges the incidence of DM at the time of diagnosis in patients with HNSCC is 7% (Dennington, Carter and Meyers, 1980). A lower incidence rate has also been reported, e.g. in an analysis of 3033 patients with mucosal head and neck cancer, the proportion of patients clinically staged as IVC, representing the patients with DM, was only 2.2% (Iro and Waldfahrer, 1998). The lung is by far the most common site for DM, followed by bone, the liver and the mediastinum (Calhoun et al., 1994; Dennington, Carter and Meyers, 1980; Garavello et al., 2006; Leon et al., 2000; Zbaren and Lehmann, 1987). The incidence of DM increases with increasing stage of the disease (Calhoun et al., 1994; Garavello et al., 2006; Leemans et al., 1993). In patients with locoregionally controlled HNSCC, the incidence of DM in the follow-up is 5-20% (Leemans et al., 1993; Leon et al., 2000; Vikram et al., 1984).

2.1.3. Second primary tumours

Because of common predisposing factors, tobacco and alcohol consumption, HNSCC may coexist with malignancies of the oesophagus and lung. If the irritant has already promoted one cancer, there is a high risk of another cancer arising from the mucosal surface under the influence of the same irritant. This concept of field cancerization was introduced in 1953 and it has been supported by recent studies, in which genetically altered cells have been found in the mucosal field surrounding the index tumour in a significant proportion of patients with

HNSCC (Slaughter, Southwick and Smejkal, 1953; Ai et al., 2001; Tabor et al., 2001; Braakhuis et al., 2002;).

Second primary tumours are defined as synchronous, if diagnosed simultaneously with the head and neck index tumour or within six months of it. Second primary tumours diagnosed later than six months after the index tumour are defined as metachronous (Brownson et al., 1973). In a meta-analysis consisting of 40 287 patients (Haughey et al., 1992), the overall incidence of second primary tumours in head and neck cancer patients was 14.2%; the incidence of synchronous second primaries was 6% (ranging 0.8%-18%). Of the second primary tumours, 35% were found in head and neck region, 25% in the lung, 9% in the esophagus, and 31% at other locations. Well in line with these figures is a recent Finnish study, in which the incidence of synchronous second primary tumours was 3.9% and the overall incidence of second primaries was 13.3% (Hujala, Sipilä and Grénman, 2005). A pulmonary second primary tumour is most often encountered in patients with laryngeal carcinoma, while oesophageal and head and neck second primaries tend to coincide with oral cavity and pharyngeal index tumours (Haughey et al., 1992; Hujala, Sipilä and Grénman, 2005; Kuriakose et al., 2002)

2.2. Pre-treatment evaluation of head and neck squamous cell carcinoma

Pre-treatment evaluation of HNSCC consists of physical examination, endoscopy and imaging studies. The goal is to establish the extent and size of the primary tumour, to detect possible regional and/or distant metastases, and to detect the possible second primary tumour in order to determine the surgical and other therapeutic options. Based on the information obtained, the disease is staged clinically according to the TNM classification system (UICC, Sobin and Wittekind, 2002), which will be discussed later.

2.2.1. Primary tumour

The primary HNSCC can usually be detected in a thorough otorhinolaryngologic physical examination supplemented by transnasal fiberoscopy if needed. Oral and oropharyngeal tumours can usually be diagnosed and biopsied under local anesthesia, while for other pharyngeal and laryngeal tumours a general anesthesia is needed to obtain adequate biopsies and to accurately evaluate the extent of the tumour on the mucosal surfaces.

CT and magnetic resonance imaging (MRI) can both be used to image the primary tumour. Both of these imaging modalities have certain advantages and disadvantages and in addition, there are many patient related aspects that need to be considered when choosing the most appropriate imaging method for an individual patient (Alberico, Husain and Sirotkin, 2004; Rumboldt et al., 2006).

2.2.2. Regional metastases

The accuracy of palpation in the assessment of the metastatic status of the cervical lymph nodes has been reported to be around 70% (Feinmesser et al., 1987; Merritt et al., 1997). Imaging of the palpably metastatic neck is performed to define the extent of tumour growth in the cervical lymph nodes, to assess the resectability of the cervical disease, and invasion of vital structures. Routine praxis is to image also the palpably non-metastatic neck to detect possible subclinical metastases.

Ultrasound (US), ultrasound-guided fine needle aspiration cytology, CT, MRI, and positron emission tomography (PET) have been used to increase the accuracy of neck assessment. The accuracy of these methods in identifying patients with cervical metastases is usually reported only slightly superior to palpation (Castelijns and van den Brekel, 2002; Curtin et al., 1998; Feinmesser et al., 1987; Hao and Ng, 2000; Merritt et al., 1997; Moreau, Goffart and Collignon, 1990; Stuckensen et al., 2000; Takes et al., 1998; van den Brekel et al., 1993; van den Brekel, 2000). The detection of metastatic lymph nodes by using anatomical imaging (CT, MRI and US) is based on criteria based on the size, shape, and morphology of the lymph nodes (Castelijns and van den Brekel, 2002). It is not possible to distinguish a lymph node harbouring a microscopically small metastasis from a non-metastatic lymph node by these criteria, however. No imaging technique can achieve good sensitivity for the detection of metastatic lymph nodes without losing high specificity (Castelijns and van den Brekel, 2002; Curtin et al., 1998). When performing ultrasound-guided fine needle aspiration cytology, it is a matter of chance, if the aspirate is obtained from the lymph node with microscopic metastasis and if so, the tip of the aspirating needle may not hit the small metastatic deposit inside the lymph node. Instead of anatomical changes, PET imaging is based on the high metabolic activity of the malignant tissue. The resolution of a modern dedicated PET-camera is 3-4mm at its best (Matsumoto et al., 2006) leaving the smallest metastases in the lymph nodes undetected. New PET-cameras have an integrated high resolution CT scanner; PET- and CT imaging can be performed at the same setting and the images can be fused. Combined PET-CT imaging has recently been reported to be more accurate in staging the cervical lymph nodes than PET or CT imaging alone (Jeong et al., 2007). It has been estimated, that about half of the necks that are false negative at palpation can be upstaged by using modern imaging techniques (Castelijns and van den Brekel, 2002).

2.2.3. Distant metastases and second primary tumours

Second primary tumours originating from the head and neck region are likely to be detected when evaluating the index tumour. Outside the head and neck region, second primary tumours are most often found in the lungs and the oesophagus. Distant metastases from HNSCC are most often found in the lungs, liver, bone and the mediastinal lymph nodes. Chest x-ray, oesophagoscopy, bronchoscopy, bone scintigraphy, abdominal ultrasound, and barium swallow have all been used in different combinations for pre-treatment evaluation of DM and

pulmonary and oesophageal second primary tumours. As the availability of CT has improved, CT of chest and upper abdomen has to a great extent replaced the former methods. CT is more sensitive in detecting pulmonary and mediastinal tumours than conventional chest x-ray (Dinkel et al., 1990; Houghton et al., 1998a; Loh et al., 2005; de Bree et al., 2000). On the other hand, the specificity of pulmonary CT in search of malignant tumours seems to be far from optimal resulting in a number of false positive findings and consequently, in numerous unnecessary further examinations (Swensen et al., 2003).

A consensus does not exist, whether screening for DM and second primary tumours by CT of the chest is indicated for all patients with previously untreated HNSCC. The results of the studies on this issue are inconclusive. Malignant findings in chest CT scans, including DM and second primary tumours, have been reported in 4-20% of HNSCC patients (Houghton et al., 1998a; Houghton et al., 1998b; Loh et al., 2005; Mercader et al., 1997; Nilssen et al., 1999; Ong et al., 1999; Reiner et al., 1997; Tan et al., 1999; de Bree et al., 2000). Subsequently, the recommendations on the routine use of chest CT are variable. Some authors recommend chest CT routinely for all patients prior to the curatively intended treatment of the HNSCC (Houghton et al., 1998a; Houghton et al., 1998b; Ong et al., 1999; Reiner et al., 1997), while others recommend it only for patients with locoregionally advanced disease or an abnormal conventional chest x-ray (Loh et al., 2005; Mercader et al., 1997; Nilssen et al., 1999; Tan et al., 1999; de Bree et al., 2000).

Whole body PET-imaging has been used as a screening method for DM and second primary tumours. Some studies show results suggestive of superiority of PET over other screening methods in the detection of DM (Brouwer et al., 2006; Schwartz et al., 2003; Wax et al., 2002). Combined PET-CT imaging may have an increasing role in the detection of DM or/and second primary tumours in the future. The main problems in PET or PET-CT imaging are the limited availability of PET-cameras, the relatively high costs, and the time-consuming imaging protocol.

2.2.4. Staging

After the clinical assessment and imaging studies eventually including fine needle aspiration cytology, malignant head and neck tumours are staged clinically according to the TNM classification system (Sobin and Wittekind, 2002). Prognostic information related to the size of the primary tumour (T), extent of regional metastatic disease (N), and presence/absence of DM (M) is combined according to an internationally standardised system and the disease is categorised as Stage I-IV. TNM staging is the most important tool for assessing prognosis in patients with HNSCC. It enables the comparison of patient populations in different studies. However, it must be noted that there are important prognostic features (e.g. depth of infiltration, extracapsular spread) that are not included in the TNM-staging system and need to be considered separately. The TNM and stage classification for oral squamous cell carcinoma are summarized in Table 1.

Table 1. TNM and stage classification for oral cavity squamous cell carcinoma

T – Primary Tumour			
Tis:	Carcinoma in situ		
T1:	≤2cm		
T2:	>2cm to 4cm		
T3:	>4cm		
T4a:	Invades through cortical bone, deep/extrinsic muscle of tongue, maxillary sinus, skin		
T4b:	Invades masticator space, pterygoid plates, skull base, internal carotid artery		
N – Regional Lymph Nodes			
NX	Regional lymph nodes can not be assessed		
N0	No regional lymph node metastasis		
N1	Ipsilateral single ≤3 cm		
N2	N2a Ipsilateral single >3cm to 6 cm		
	N2b Ipsilateral multiple ≤6 cm		
	N2c Bilateral or contralateral ≤6 cm		
N3	>6 cm		
M – Distant Metastasis			
MX	Distant metastasis can not be assessed		
M0	No distant metastasis		
M1	Distant metastasis		
Stage Grouping			
Stage 0	Tis	N0	M0
Stage I	T1	N0	M0
Stage II	T2	N0	M0
Stage III	T1,T2	N1	M0
	T3	N0,N1	M0
Stage IVA	T1,T2,T3	N2	M0
	T4a	N0,N1,N2	M0
Stage IVB	Any T	N3	M0
	T4b	Any N	M0
Stage IVC	Any T	Any N	M1

Modified from: TNM classification of malignant tumours. 6th edition. Sobin L.H., Wittekind Ch, editors. Wiley-Liss, New York, 2002.

2.3. Clinicopathological features of oral cavity tumours having predictive value

2.3.1. Features related to the primary tumour

The size of the primary tumour, reflected by the T-category, is an important determinant of prognosis. The T-category is determined by the greatest tumour diameter or extension of the tumour to neighbouring tissues (Sobin and Wittekind, 2002). The larger the tumour is, or the greater the T-category, the larger is the proportion of the patients presenting with cervical or/and distant metastases and the worse is the survival (Baatenburg de Jong et al., 2001; Byers et al., 1998; Calhoun et al., 1994; Garavello et al., 2006; Le Tourneau et al., 2005). The 5-year survival rates for various T-categories in all HNSCC patients has been reported to be as follows: T1: 72%, T2: 52%, T3: 36% and T4: 26% (Baatenburg de Jong et al., 2001). The incidence of DM in the follow-up has been reported to be only 0.3 % for patients with T1 tumours, while 21% of the patients with T4 tumour developed DM (Garavello et al., 2006). Recently, the size of the tumour expressed by tumour volume has been reported to be the more accurate prognostic factor than size expressed by tumour diameter (Le Tourneau et al., 2005).

In SCC of the oral cavity, the thickness of the primary tumour, represented by tumour thickness or depth of infiltration, is widely accepted to be a predictor of nodal metastasis and survival. The thicker the tumour is, the larger is the proportion of the patients presenting with cervical nodal disease and the poorer are the survival rates (Al-Rajhi et al., 2000; Asakage et al., 1998; Brown et al., 1989; Byers et al., 1998; Fakih et al., 1989; Fukano et al., 1997; Gonzalez-Moles et al., 2002; Kurokawa et al., 2002; O-charoenrat et al., 2003; Okamoto et al., 2002; Po Wing Yuen et al., 2002). In many studies, the thickness of the primary oral cavity tumour has been the only histopathological parameter having a significant predictive value (Al-Rajhi et al., 2000; Asakage et al., 1998; O-charoenrat et al., 2003; Po Wing Yuen et al., 2002; Spiro et al., 1986). In a recent review of 55 studies, tumour thickness was found to be a reliable parameter for the prediction of sub-clinical regional lymph node involvement in oral cancer patients (Pentenero, Gandolfo and Carrozzo, 2005). There is, however, great variation in the cut-off thickness having clinical significance, i.e. thickness that can be used as a limit to indicate patients with a high risk of cervical metastases and poor survival. This critical thickness ranges from 1.5 to 10 mm in different studies, being most often 4 mm. Comparison of different studies is hampered by differences in measurement technique of the tumour thickness, or depth of infiltration. Measurements from the level of normal mucosa (i.e. depth of infiltration), from the level of basal membrane (i.e. depth of infiltration), or from the surface of the tumour (i.e. tumour thickness) to the deepest portion of the tumour infiltration have been used. In some studies the method of measurement has not been defined. Some of the studies demonstrating the relationship between tumour thickness, nodal involvement and survival are collated in Table 2.

Table 2. Studies on predictive value of tumour thickness

Study	n	Tumour site	Clinical stage	Tumour thickness (mm)	Nodal involvement (%)	Survival (%)
Fakih et al 1989	70	tongue	I-II	<4	14	81
				>4	73	43
Asakage et al. 1998	44	tongue	I-II	<4	12	ND
				>4	70	
Kurokawa et al. 2002	50	tongue	I-II	<4	3	ND
				>4	38	
Okamoto et al. 2002	59	tongue	I-II	<4	9	ND
				>4	45	
Po Wing Yuen et al. 2002	72	tongue	I-II	<3	8	100
				3-9	44	76
				>9	53	66
O'Brien et al. 2003	145	oral cavity	I-IV	<4	8	100
				>4	48	74
O-charoenrat et al. 2003	50	tongue	I-II	<5	16	95
				>5	64	32,5

Abbreviations: ND; not defined

The results of many recent studies show poor correlation between the histological grade of the tumour, expression of the degree of differentiation, and survival (Bryne et al., 1992; Le Tourneau et al., 2005; O-charoenrat et al., 2003; Po Wing Yuen et al., 2002; Woolgar, 2006). Grading of the whole tumour seems to have little additional value as a prognostic factor in HNSCC patients. However, grading of specifically the deep invasive front of the tumour seems to have a prognostic value, the pattern of the tumour invasion being the component of the grading system having most prognostic significance (Bryne et al., 1992; Fukano et al., 1997; Sawair et al., 2003; Odell et al., 1994).

Additional histopathological factors of the primary tumour that have been linked to increased risk of regionally metastatic disease and/or reduced survival are perineural invasion (Brown et al., 1989; Clark et al., 2006), microvascular invasion (Close et al., 1987), and lymphovascular invasion (Brown et al., 1989).

The width of the uninvolved resection margin in the surgically removed HNSCC has been shown to be of high prognostic value (Binahmed, Nason and Abdoh, 2006; Loree and Strong, 1990; Sutton et al., 2003). It has been demonstrated that when excision of a T1 or T2 oral

tumour is complete with good margins (>5 mm), local recurrences are rare and not linked to any other histopathological parameter (van Es et al., 1996). Patients with histopathologically close or involved resection margins have more frequently local recurrences and worse survival compared to patients with clear margins. Increasing tumour size leads more often to close or involved resection margins (Loree and Strong, 1990; Sutton et al., 2003), which is presumably due to technical difficulties in achieving good margins when resecting large tumours. Involved resection margins have been linked to histological indicators of aggressive disease behaviour and according to Sutton et al. (Sutton et al., 2003) can thus be regarded as an indicator of aggressive disease.

2.3.2. Features related to regional metastases

The extent of cervical metastatic growth, reflected by the N-stage, predicts survival. The cure rates drop roughly to half when macroscopic cervical metastasis is present at the time of diagnosis (Alvi and Johnson, 1996; Baatenburg de Jong et al., 2001; Teichgraber and Clairmont, 1984). The higher the N-stage, the larger is the proportion of the patients presenting with DM and the worse is the survival (Alvi and Johnson, 1997; Baatenburg de Jong et al., 2001; Garavello et al., 2006; Le Tourneau et al., 2005; Leon et al., 2000). The 5-year survival rates for HNSCC patients staged N0, N1, N2 and N3 has been reported to be 63%, 32%, 26%, and 11%, respectively (Baatenburg de Jong et al., 2001). The incidence of DM in the follow-up has been reported to range from 2.5% in patients with N0 neck to 29.5% in patients diagnosed as having N3 neck disease (Garavello et al., 2006).

Tumour extension outside the lymph node capsule, i.e. extracapsular spread, has been shown to be a powerful predictor of regional recurrence, DM, and poor survival (Alvi and Johnson, 1996; Alvi and Johnson, 1997; Garavello et al., 2006; Leemans et al., 1993; Leon et al., 2000; Myers et al., 2001). Some studies even suggest that there is no significant difference in survival between patients with pathologically confirmed cervical metastatic disease (pN+) without extracapsular spread and patients with pathologically non-metastatic neck (pN0), whereas the presence of extracapsular spread is associated with increased regional and distant recurrences and poor survival (Johnson et al., 1981; Jose et al., 2003). Extracapsular spread is encountered even in patients with clinically N0 disease, as demonstrated in a study consisting of 109 patients undergoing elective neck dissection (END) for clinically N0 disease (Alvi and Johnson, 1996). Of the patients, 34% had occult metastases and in half of them, extracapsular spread was detected. Also in that study, extracapsular spread was a strong indicator of recurrent disease and poor prognosis.

In addition, the number of metastatic lymph nodes (Alvi and Johnson, 1997; Kowalski et al., 2000; Le Tourneau et al., 2005; Leemans et al., 1993) and anatomical level of node involvement (Kowalski et al., 2000) have been reported to predict outcome of patients with HNSCC; the risk being higher for patients with multiple metastatic nodes, involved nodes at levels IV and V, and/or contralateral or bilateral metastatic nodes. In a study by Leemans and

colleges (Leemans et al., 1993), cervical metastases meant a twofold risk and histopathologically detected extracapsular tumour growth in cervical lymph nodes a threefold risk of DM; an exceptional risk of DM was found in patients with more than three metastatic cervical lymph nodes, of which 47% developed DM during the follow-up.

2.4. Elective treatment of the cervical lymph nodes

2.4.1. Occult metastases

Occult metastasis refers to a metastasis that can not be detected by clinical or imaging examination including fine needle aspiration cytology. As discussed previously, the imaging modalities currently in use cannot detect all early metastases. At present the most accurate method for assessing a clinically non-metastatic neck is END and histopathological analysis of the neck dissection specimen, which does not guarantee the detection of all small metastatic deposits in the lymph nodes, however. For standard histopathological analysis, lymph nodes from a neck dissection specimen are usually only cut in half and a single 5- μ m section from each lymph node is examined. Consequently, most of the nodal tissue is never examined by the pathologist and microscopically small metastases can be missed. A single 5- μ m section represents only 1/2000 of the total width of a lymph node 1 cm in diameter. By employing extensive histopathological protocols including serial sectioning of the lymph nodes and immunohistochemical stainings, microscopically small metastatic deposits are found in 8- 15% of patients initially staged pN0 after conventional histopathological examination of the neck dissection specimen (Ambrosch and Brinck, 1996; Ferlito, Shaha and Rinaldo, 2002; Hamakawa et al., 2000; van den Brekel et al., 1992). However, all lymph nodes can not be examined in such detail because of the resulting unacceptably heavy workload for the pathologist and high costs.

The presence of occult cervical metastasis can be predicted by the size and histopathological features of the primary tumour as discussed in the previous chapter.

2.4.2. Clinically N0 neck – elective treatment or observation?

Elective neck treatment refers to treatment of the cervical lymph nodes performed as a precaution, because cervical nodal metastases can not be excluded. Elective neck treatment usually means END in conjunction with the resection of the primary tumour, though RT can also be used, if the primary tumour is treated by RT. The advantage of END is that it allows pathologic staging of the cervical lymph nodes and provides important histopathological information (i.e. presence of metastatic lymph nodes, extracapsular spread) on the patients at greatest risk and in need of postoperative radiation or chemoradiation. The morbidity related to unilateral END is usually considered reasonable (Laverick et al., 2004).

The appropriate treatment regimen of clinically N0 neck in patients with HNSCC has been a subject of much debate. It is a commonly accepted principle to treat the neck electively when the risk of occult metastasis is estimated to exceed 15-20%; otherwise the neck is left under close follow-up and treated therapeutically, if metastases have developed at follow-up (van den Brekel et al., 1993; Wei et al., 2006). This approach results in considerable over-treatment and on the other hand, occult cervical metastases of some patients are initially left untreated.

The real benefits of elective neck treatment remain unclear. There are only three prospective randomised studies comparing END with observation of the neck and therapeutic neck treatment when necessary (Fakih et al., 1989; Kligerman et al., 1994; Vandenbrouck et al., 1980). The results of these studies and the retrospective studies on this topic have been inconclusive. Some of the studies have failed to reach statistically significant differences in survival between patient groups with either electively or therapeutically treated neck nodes (Duvvuri et al., 2004; Fakih et al., 1989; Vandenbrouck et al., 1980; Yii et al., 1999) while in other studies there has been a significant survival benefit in favour of the elective neck treatment (Dias et al., 2001; Haddadin et al., 1999; Kligerman et al., 1994). The observed patients tend to have markedly more regional recurrences (Dias et al., 2001; Duvvuri et al., 2004; Spiro, Spiro and Strong, 1986) and there is evidence that cervical nodal disease is more difficult to cure when it presents clinically (Dias et al., 2001; Duvvuri et al., 2004; Haddadin et al., 1999; Kowalski, 2002; Yii et al., 1999). It seems probable that the small sample sizes of many studies do not offer sufficient power to demonstrate the potentially existing small difference in survival between patients who have had elective neck treatment and those who have not.

2.5. Sentinel lymph node biopsy

2.5.1. General considerations

Sentinel lymph node (SLN) is the first lymph node to receive lymphatic drainage from the primary tumour site and is therefore thought to be the first lymph node to harbour a lymphogenic metastasis. Therefore, according to the theory, in patients with clinically N0 disease the presence or absence of metastases in the lymphatic basin can be assessed by a selective biopsy and assessment of the SLN(s). If the SLN(s) is free of tumour, the whole lymphatic basin can be considered to be free of tumour and does not need to be treated.

The concept of SLN was first described in 1960 by Gould et al., who successfully used frozen section analysis of a lymph node in a certain location near the caudal end of the parotid gland to guide in the decision-making whether to perform a neck dissection or not in patients with parotid malignancies (Gould et al., 1960). In 1977 Cabanas described the use of preoperative lymphangiograms to identify inguinal lymph nodes at greatest risk for metastatic growth in patients with penile carcinoma (Cabanas, 1977). In 1992 Morton et al. first described the

method of intraoperative localization of the SLN(s) using blue dye in patients with early stage melanoma (Morton et al., 1992). In this preliminary study, SLN biopsy detected the patients with occult metastases with high accuracy. In 1994, van der Veen et al. introduced the intraoperative use of handheld gamma probe, which is currently the most important method for SLN identification (van der Veen et al., 1994). Since then, validation studies have demonstrated that SLN status accurately reflects the status of the whole lymphatic basin in breast cancer and melanoma (Krag et al., 1998; Lyman et al., 2005; Morton et al., 1999; Morton et al., 2006). Sentinel lymph node biopsy has become a standard of care in the treatment of these tumours sparing the SLN-negative patients the morbidity of more comprehensive lymphadenectomies. In recent years, the concept of SLN biopsy has been applied to many other malignant tumours, such as oesophageal, gastric, colorectal, and lung tumours (Kitagawa et al., 2002a; Kitagawa et al., 2002b; Lee et al., 2006; Rzyman et al., 2006). It seems probable that SLN biopsy will have an increasing role in the surgical treatment of malignant tumours in the future.

2.5.2. Technique

Lymphoscintigraphy, a handheld gamma-probe and blue dye can be used to identify the SLN(s) (Stoeckli et al., 2005). Preoperatively, the SLN(s) is identified by lymphoscintigraphy, which is typically performed on the day before surgery. Radioactive tracer is injected peri- or intratumorally and lymphoscintigraphic images are obtained in anterior and lateral projections. The tracer accumulates in the SLN(s), which can then be seen on lymphoscintigraphy. During lymphoscintigraphic imaging the location of the SLN(s) can be marked on the skin to help anatomic orientation of the surgeon. At operation, a handheld gamma-probe is used to identify the SLN(s), which emits gamma-rays because of accumulation of radiotracer. Blue dye is often used in addition to gamma detection to enhance the identification of the SLN(s). Before the operation, blue dye is injected around the tumour and shortly thereafter the lymphatic channels and SLN(s) become stained by blue colour. Different modifications of the technique are used. The minimal requirement for SLN biopsy is the peritumourally injected radiotracer and the use of a handheld gamma-probe (Stoeckli et al., 2005).

It is generally accepted that histopathological analysis of SLNs has to be more comprehensive than standard examination of lymph nodes from a lymphadenectomy specimen, because missing a small tumour deposit in a SLN could result in a wrong decision not to treat the whole lymphatic basin. It has been recommended that SLNs free of tumour cells after conventional histopathological examination (sections at 1-2 mm intervals, H&E staining) should undergo serial sectioning of the whole SLN at 150 μ m intervals and immunohistochemical stainings to rule out the possibility of micrometastasis in HNSCC patients (Stoeckli et al., 2005). In breast cancer, according to a recent recommendation (Lyman et al., 2005), the SLNs are cut into sections no thicker than 2.0 mm parallel to the longest axis of the lymph node and one or two step sections are cut at 200- to 500 μ m

intervals into the block in addition to the top level. Immunohistochemistry is often used in the histopathological analysis of SLNs in breast cancer. However, it is not recommended as a required part of SLN evaluation in breast cancer, because the clinical significance of the small clusters of cancer cells detected by this method is unclear (Lyman et al., 2005). Intraoperative SLN analysis (allowing immediate axillary dissection) can be used in breast cancer. Then, however, 25% of patients with positive nodes will be detected only after the assessment of the permanent sections (Lyman et al., 2005).

2.5.3. Sentinel lymph node biopsy in head and neck squamous cell carcinoma

Sentinel node biopsy was first introduced in the treatment of HNSCC in late 90's (Koch et al., 1998; Shoaib et al., 1999). Since then, SLN biopsy has been found to be a sensitive method (>90% detection rate) for finding occult metastases in patients with oral/oropharyngeal squamous cell carcinoma in many studies (Paleri et al., 2005; Ross et al., 2002; Ross et al., 2004; Stoeckli et al., 2005; Tschopp et al., 2005). Tumours in these locations are suitable for the method, because of their accessibility to injections without general anaesthesia. In some studies the method has been applied also to hypopharyngeal and laryngeal tumours by performing the injections in general anesthesia with immediate localisation and removal of the SLN(s) (Werner et al., 2004; Werner, Dunne and Davis, 2005).

The pooled data presented at the Second International Conference on Sentinel Node Biopsy in Mucosal Head and Neck Cancer (Zurich, Switzerland, 2003) consisted of 379 patients with clinically N0 disease. In 97% of the patients the SLN(s) could be identified, and the overall sensitivity of SLN biopsy to detect occult disease was 90%; the negative predictive value of a negative SLN for the remaining neck was 96% (Stoeckli et al., 2005). In a study by Ross et al (Ross et al., 2004), 134 patients at six institutes diagnosed with T1/T2N0 oral/oropharyngeal cancer were included. At least one SLN could be identified in 93% cases and the overall sensitivity of SLN biopsy to detect occult metastatic disease was 93%. In that study, the sensitivity of SLN biopsy for floor of mouth tumours was 80% versus 100% for tumours in other oral and oropharyngeal locations. The lower success rate for floor of the mouth tumours is presumably due to the close proximity of the floor of mouth tumours to the SLN(s) located in the submandibular region (Level I). The radioactivity of the closely situated primary tumour site interferes with the identification of the SLN(s) and one or more SLN(s) may be missed.

It remains to be seen, whether SLN biopsy will have an effect on the DSS of patients diagnosed with oral cavity SCC. Obviously, the DSS rate can not be improved in patients who undergo a SLN biopsy instead of END. The DSS rate for these patients should remain at the same level as it now is for patients who receive END, but with reduced morbidity. In patients with a very small primary tumour who do not receive elective neck treatment according to current treatment protocols, a slight improvement in DSS rate may theoretically be achieved. Since the morbidity of SLN biopsy is minimal, it can be performed for these low risk patients

instead of only observing their neck status. Then, the few patients with cervical metastasis in this patient group will get neck treatment while their neck disease is still at an occult stage, provided that SLN biopsy proves to be an accurate staging method.

Ideally, SLN biopsy would replace END as a staging procedure for the cervical lymph nodes in patients with T1-2N0 oral and oropharyngeal squamous cell carcinoma. More extensive lymphadenectomies could then be targeted specifically to patients with true metastatic disease and the rest of the patients would avoid the morbidity related to neck dissection. Before that, prospective multicenter validation studies currently conducted in Europe and in the USA must verify that SLN status accurately reflects the metastatic status of the rest of the cervical lymph nodes.

3. Aims of the study

The general objective of this study was to improve the pre-treatment staging evaluation of patients with newly diagnosed HNSCC and through improved staging of the neck to choose the appropriate treatment for an individual patient. Specifically focused was the evaluation and treatment of patients with oral cancer with no clinical and radiological signs of metastatic disease.

The specific aims of the present study were:

1. To evaluate the benefit of routine screening for DM or/and second primary tumours with chest and abdominal CT in patients with newly diagnosed HNSCC (Study I).
2. To improve the pre-treatment evaluation of the cervical lymph nodes and to better target elective neck treatment for patients with oral cavity cancer (Studies II-V).
 - a) by analysing the treatment results of patients diagnosed with stage I-II oral tongue cancer with special reference to the potential effect of the chosen neck treatment regimen on the treatment outcome (Study II).
 - b) by analysing whether histopathological features of the primary tumour can be used to identify patients at high risk and at need for treatment of the neck (Study III).
 - c) by examining the feasibility and accuracy of SLN biopsy in staging the clinically negative neck in patients with oral cavity cancer (Studies IV and V).

4. Patients and methods

4.1. Patients and study designs

All patients included in Studies I-V were diagnosed and treated at the Helsinki University Central Hospital, which is a tertiary referral center covering an area of 1.4 million inhabitants.

4.1.1. Study I

CT scanning of the chest and abdomen was performed for 100 consecutive patients diagnosed with HNSCC between June 2001 and July 2003 and the findings were analysed. Of specific interest was the impact of possible findings on the management of these patients. The patients who had newly diagnosed T2-T4 HNSCC (T3-T4 for glottic and lower lip cancers), a cervical metastasis of any HNSCC primary tumour or from unknown origin, and who were considered to be suitable for treatment with curative intent were prospectively enrolled. Patients with T1 tumours (T1 and T2 for lower lip cancer) were excluded because of the very low risk of DM.

After an intravenous injection of contrast medium (1.5 ml/kg; iopentol 300 mg iodine/ml, rate 2 ml/s), the patients underwent CT scanning with an MX 8000 QUAD (Philips, Medical Systems, Eindhoven) from the lung apices to the inferior poles of kidneys on 25 patients and from the lung apices to the pelvic floor on 75 patients. The images were interpreted and viewed by two experienced radiologists at window settings appropriate for the assessment of soft tissue (level, 40-50 HU; width, 300-350 HU) and for the assessment of lung tissue (level, 750 HU; width, 1200 HU). The follow-up of the patients was carried out according to our standard protocol (i.e. every 2-3 months during the first two years and every 4-6 months thereafter).

The median age of the 100 patients enrolled into the study was 60 years, ranging from 36 to 93 years. The primary tumour sites included the oral cavity (n= 20), pharynx (n= 54), larynx (n= 20), and the maxillary sinus (n= 1). The clinical stage of these patients was as follows: Stage II: n=10, Stage III: n=19, and Stage IV: n=66. In five patients with cervical metastases of SCC, the location of the primary tumour was unknown. Of the patients, 34 had N0 necks and 66 had N+ necks after clinical assessment and imaging studies.

4.1.2. Study II

In Study II, the treatment results of patients diagnosed with a clinically T1/T2N0 SCC of the oral tongue between 1992 and 2002 were retrospectively analysed. Patients with a minimum of 24 months follow-up data or until death were included. Eighty patients were eligible for inclusion (39 females, 41 males). The median age of the patients was 57 years, ranging from 23 to 96 years. The median follow-up time was 55 months, ranging from 5 to 150 months. The clinical T classification was as follows: T1: n=40 (50%), T2: n=40 (50%). All the

patients had been surgically treated with curative intent. The hospital records were reviewed and data on patient characteristics, histopathology, treatment, and follow-up were collected. The date and cause of death was provided by Statistics Finland, the national agency for population statistics.

The decision to give or not to give elective neck treatment was based on the risk of metastatic disease assessed at our multidisciplinary tumour board. Two groups of patients were formed according to the chosen neck treatment regimen: patients who had had elective neck treatment (n=46, the ELNT group) and patients who had been observed for their neck status (n=34, the OBS group). Elective neck treatment consisted of END in 44 patients followed by postoperative RT in 35 patients. Two patients had received elective neck RT only. All patients who had received RT to the neck had also had postoperative RT to the primary site. A comparison of these two groups with respect to OS, DSS, and recurrency was performed. The result of salvage treatment were analysed. The incidence of occult cervical metastases was calculated. In patients who had not had an END, occult neck disease was defined as a histologically proven neck metastasis detected during the follow-up without failure at the primary site. Cervical metastases in patients with recurrent primary tumour were not considered occult metastases, because the nodal spread may have occurred after the initial treatment. In patients who had had an END, occult neck disease was defined as presence of microscopic disease on the histopathological examination of the neck dissection specimen.

4.1.3. Study III

The clinicopathological data of 73 patients diagnosed with SCC of the oral tongue were retrospectively reviewed. The series consisted of the same patient population as in Study II, except that the patients without original histopathological material available for review were excluded, resulting in 73 eligible patients (37 females, 36 males). The median age of the patients was 59 years, ranging from 23 to 95 years. The T classification was as follows: T1: n= 35 (48%), T2: n= 38 (52%). All patients had been surgically treated with curative intent. In addition to the data collected from the hospital records, the histopathological paraffin sections of each patient were reviewed by a single pathologist experienced in head and neck pathology. The depth of infiltration, grade, greatest diameter, and mode of invasion of the primary tongue tumour were re-assessed. The mode of invasion was classified into one of three categories: pushing-border, diffuse, destructive. Depth of infiltration was measured from the level of the adjacent normal mucosal surface to the deepest portion of the tumour invasion in the tongue musculature. Measurements were expressed with an accuracy of 0.1 mm. Only re-assessed histopathological data were used in the analysis with the exception of tumour diameter. Since reconstruction and measurement of tumour diameter is less reliable afterwards than the original measurement, the re-assessed data on tumour diameter was used only when original data were not available. According to the pathologically analysed tumour diameter, the tumour was classified as pT1 (≤ 20 mm) or pT2 (21-40 mm). The ability of histopathological variables to predict occult nodal metastases, local recurrence and survival

was analysed. Occult metastasis was defined as in Study III. One patient with prophylactic neck RT only was not taken into account when assessing the correlations between histopathological parameters and occult metastases.

4.1.4. Study IV

Study IV was a prospective study aiming at assessing the feasibility and accuracy of SLN biopsy in patients with oral and oropharyngeal SCC. Patients with previously untreated SCC of the oral cavity or oropharynx (T1-T3) with no evidence of metastatic lymph nodes (N0) as assessed by palpation and MRI or CT, and for whom END was planned as part of the treatment, were eligible for this study. Patients with oropharyngeal tumour that was not accessible to injection without general anesthesia were excluded. Forty-six consecutive patients (19 females, 27 males) were enrolled between January 2002 and September 2005. The median age of the patients was 57 years, ranging from 32 to 84 years. Forty-five patients had SCC of the oral cavity (25 tongue, 11 floor of mouth, five gingiva of the mandible, two palate and two gingiva of the maxilla) and one patient had SCC of the oropharynx (tonsilla). Eighteen patients had T1N0, 21 patients T2N0, and seven patients T3N0 disease.

Lymphoscintigraphy, blue dye, and a hand-held gamma probe were used to identify the SLNs. Lymphoscintigraphy was performed the day prior to surgery using technetium ^{99m}Tc labelled colloidal human serum albumin (either Nanocoll® or Albures®, Nycomed Amersham, High Wycombe, UK) injected in the close proximity of the primary tumour. During lymphoscintigraphy, the locations of the SLNs were marked on the skin. Prior to the operation, 1.0-2.0 ml of Patent Blue® dye (Laboratoire Guerbet, Aulnay-Sous-Bois, France) was injected peritumourally. Before neck dissection, the SLNs were identified. All radioactive lymph nodes were identified using a hand-held gammaprobe (Neo2000 Gamma Detection System®, Neoprobe Corp, Columbus, OH, USA) and removed. All lymph nodes with blue stain, that could be identified, were removed. A lymph node was considered to be the SLN if its activity count was at least ten times the count of the background and/or if it was blue-coloured. After removal of the SLNs, a standard END (levels I-III or I-IV) was performed and eventually the primary tumour was resected.

The metastatic status of the SLNs and the other lymph nodes in the neck dissection specimen were compared according to a standardised protocol. For histopathological analysis, six-micrometer sections were cut from SLNs at 1-2 mm intervals, and from the other lymph nodes at intervals of not more than 5 mm. A standard hematoxylin and eosin (H&E) staining was performed and the sections were examined in light microscopy for possible metastasis. If false negative SLNs (i.e. metastatic non-SLN without metastasis in a SLN) were encountered, they were further cut taking at least two sections at 150 µm intervals. One of these was stained for routine H&E and the other for immunohistochemical detection of cytoplasmic cytokeratins to further analyze the metastatic tumour status of these SLNs. For

immunohistochemistry, sections were treated with a mixture of polyclonal antibodies to high-molecular and low-molecular weight cytokeratins (antibody AE1/AE3).

4.1.5. Study V

Sentinel lymph node biopsy was applied to 13 consecutive patients (9 females, 4 males) diagnosed as having a small (T1N0) oral/oropharyngeal SCC with no signs of metastatic lymph nodes as assessed by palpation and MRI or CT, and for whom elective neck treatment was not indicated according to the standard treatment protocol of Helsinki University Central Hospital because of low risk of occult metastasis. The median age of the patients was 65 years, ranging from 30 to 84 years. The primary tumour was located as follows: oral tongue (n=11), soft palate (n=1), buccal mucosa (n=1). In all patients, the primary tumour was clinically and pathologically classified as T1.

Lymphoscintigraphy, blue dye, and a hand-held gamma probe were used to identify the SLNs. At operation, the SLNs were removed and the primary tumour was resected. All SLNs were cut in six-micrometer sections at 1-2mm intervals. A standard H&E staining was performed and the sections were examined for possible metastases. To detect micrometastases, immunohistochemical staining for cytokeratin AE1/ AE3 was used on sections that were negative for malignant cells after examination with H&E. A neck dissection was performed on patients having a metastasis detected in a SLN.

The follow-up of the patients was based on clinical visits according to our standard protocol described in chapter 4.1.1.

4.2. Statistical methods

In Study II, OS and DSS rates were calculated by computerized software package (SPSS, Version 9.0, Chicago, IL) using Life-Table analysis. Correlations between neck treatment groups were performed using Pearson's Chi-square analysis.

In Study III, Wilcoxon rank-sum test was used in the comparison of two populations. The Pearson coefficient of correlation ρ was used to illustrate the dependency between the depth of infiltration and the greatest diameter. The Kaplan-Meier method was applied in the analysis of DSS of two populations. The difference of two survival curves was statistically tested using log-rank test. The analysis of separating patients with and without occult metastases based on the depth of infiltration was carried out using the bootstrap resampling procedure.

The results were considered significant when the p value was <0.05.

4.3. Ethics

The Research Ethical Committee of Helsinki University Central Hospital approved all study protocols used. According to the study protocols, an informed consent was requested from patients included in studies I, IV and V. Due to the retrospective character of the study and death of a considerable proportion of patients, an informed consent was not obtained from patients included in the Studies II and III.

5. Results

5.1. CT of chest and abdomen (Study I)

In two of the 100 patients examined in Study I, an obviously malignant finding was detected in the CT of the chest and the abdomen: one patient with laryngeal cancer had mediastinal metastatic lymph nodes and one patient with tonsillar cancer had a second primary tumour or a metastasis in the upper lobe of the left lung. These findings resulted in a decision not to treat these patients with surgery. In addition, there was one patient with laryngeal cancer, in whom the CT showed a large aortic aneurysm that had to be operated on before the treatment of the laryngeal cancer. Therefore, in three patients (3%) the CT of the chest and abdomen had an impact on the treatment of the HNSCC. Additional findings in CT were detected in 17 patients. Two additional patients had an aortic aneurysm, one of which had later to be treated with a stent. Fifteen patients had unspecific thoracic or abdominal findings requiring further investigations or close follow-up. Further examinations were performed on 11 of these patients. These further examinations did not reveal any significant findings. The abdominal CT was negative for metastatic HNSCC in all patients.

The follow-up time ranged from 1-31 months with a median of 13 months. Five patients were lost to follow-up. In six patients (6%), DM developed during the follow-up. One of these patients initially had a small unspecific pulmonary consolidation, which turned out to be a metastasis. Five other patients with DM developing during the follow-up did not have any findings in the initially performed CT scans of the chest and abdomen.

5.2. Elective neck treatment versus observation (Study II)

The effect of the chosen neck treatment regimen (observation or elective neck treatment) on the regional recurrences and survival in 80 patients diagnosed with T1-T2N0M0 SCC of the oral tongue was analysed in study II. The OBS group consisted of 34 patients (13 males, 21 females). Twenty-eight patients (82%) had a T1 primary tumour, while 6 patients (18%) had a T2 tumour in the OBS group. A total of 15 patients (44%) relapsed at the primary site and/or neck and 8 (53%) of the patients who relapsed died of the disease. The pattern of locoregional recurrences is demonstrated in Table 3. Distant metastases, in the liver and in the lung, were detected in 2 patients, who also had a locoregional recurrence diagnosed previously. The 3- and 5-year OS rates were 79% and 66% (Figure 3) and the 3- and 5-year DSS rates for this group were 81% and 77%, respectively (Figure 4).

The ELNT group consisted of 46 patients (28 males, 18 females). Twelve patients (26%) had T1 primary tumour, while 34 patients (74%) had T2 tumour in the ELNT group. Forty-four patients had undergone END and metastatic lymph nodes were found in 15 (34%) patients (pN1: n=12, pN2b: n=3). A total of nine patients (20%) relapsed at the primary site and/or neck and eight of these patients (89%) died of disease. Five of the six patients with recurrence

in cervical lymph nodes had had pathologic lymph nodes in the neck dissection specimen. Distant metastases were not encountered in this cohort. The 3- and 5-year OS rates for this group were 76% and 63% (Figure 3) and the 3- and 5-year DSS rates were 82% and 82%, respectively (Figure 4).

The 5-year DSS was better in the ELNT group (82% versus 77%) but there were no statistically significant differences in OS or DSS between the study cohorts.

Table 3. Locoregional recurrences and salvage treatment

	No. in group (%)		
	OBS (n=34)	ELNT (n=46)	Total (n=80)
Site of failure			
Primary site	3 (9)	3 (7)	6 (8)
Ipsilateral neck	7 (21)	4 (9)	11 (14)
Contralateral neck	0	0	0
Bilateral neck	1 (3)	0	1 (1)
Primary site + neck	4 (12)	2 (4)	6 (8)
Total	15 (44)	9 (20)	24 (30)
Salvage treatment			
Yes	10	4	14
No	5	5	10
Salvaged	7	1	8

Abbreviations: OBS, Patients who did not have elective neck treatment; ELNT, Patients who had elective neck treatment

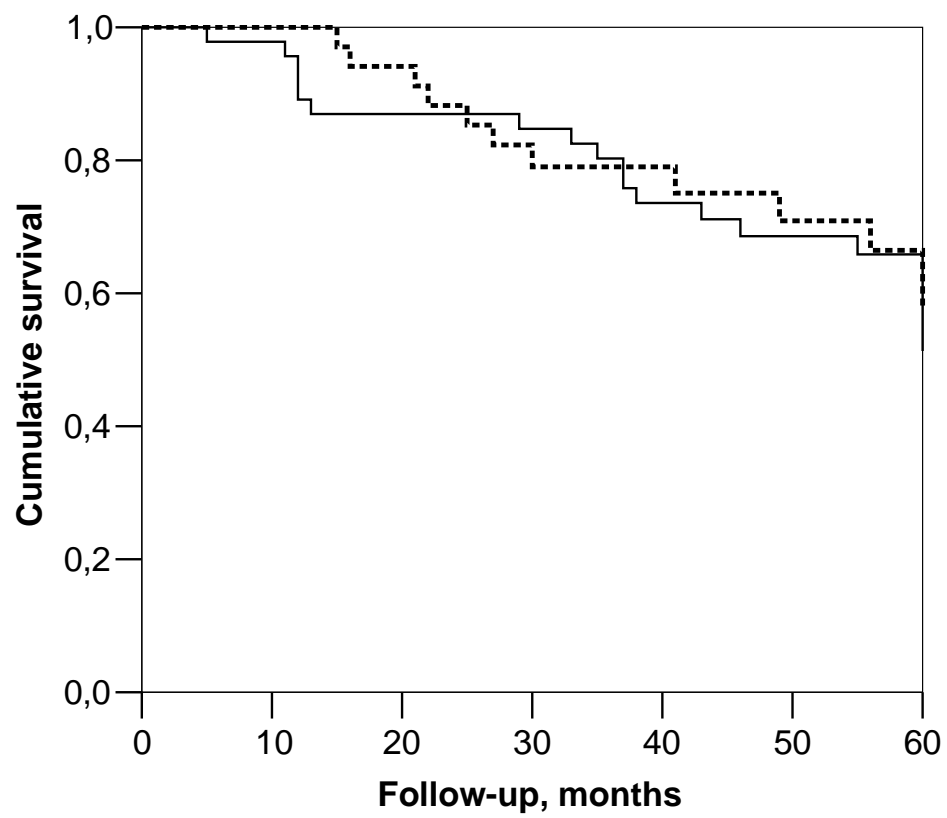


Figure 3. Overall survival of the observation group (dotted line) and the elective neck treatment group (solid line).

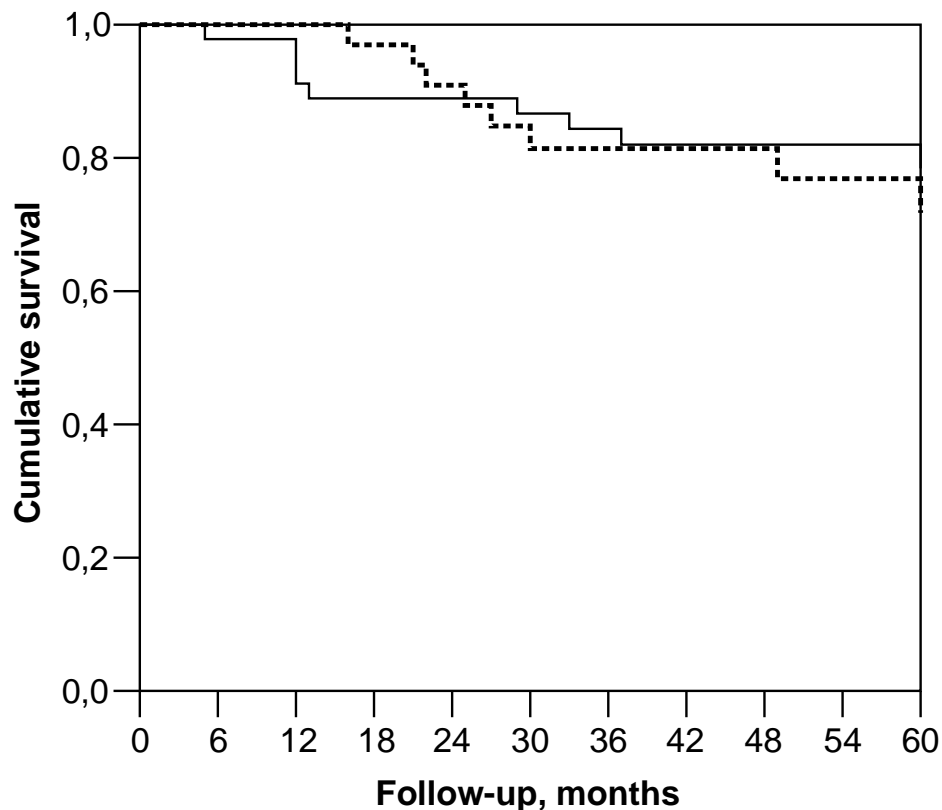


Figure 4. Disease specific survival of the observation group (dotted line) and the elective neck treatment group (solid line).

When the effect of the neck treatment on regional control was assessed, the patients with recurrence at the primary site were excluded, because nodal spread may have occurred after the initial treatment. After exclusion of patients with recurrence at the primary site, there were eight out of 27 patients with regional recurrence in the OBS group and four out of 41 patients in the ELNT group. The difference in the regional failure rate between the study groups was statistically significant ($p=0.035$).

5.3. Incidence of occult metastases (Studies II and III)

The incidences of occult metastasis in patients with clinically T1N0 and T2N0 tongue tumours were 24% (9/38) and 35% (14/40), respectively (Study II). The incidences of occult metastases for pathologically T1 and T2 tumours were 23% (11/48) and 50% (12/24), respectively (Study III).

5.4. Results of salvage treatment (Study II)

The results of salvage treatment of patients with locoregionally recurrent tongue cancer were analysed in Study II (Table 3). Salvage treatment could be offered for 10 out of 15 patients (67%) with recurrent disease in the OBS group resulting in cure in seven (47%) patients. In the ELNT group, salvage treatment with curative intent could be offered for four out of nine patients (44%) with recurrence and cure was achieved in one patient (11%). Of all 24 patients who had locoregional recurrence during the follow-up, eight (33%) were successfully salvaged.

5.5. Survival of patients with early tongue cancer (Studies II and III)

In Study II, the 3- and 5-year OS rates for the whole patient series consisting of 80 patients diagnosed with clinically T1/T2NO oral tongue cancer were 74% and 43%. The 3- and 5-year DSS rates for these patients were 82% and 79%, respectively. The 5-year DSS rates for patients with T1 and T2 tongue tumours were 83% and 76%, respectively.

In Study III, the 5-year DSS rates for patients with and without occult metastases were 65% and 91%, respectively ($p<0.01$). The 5-year DSS rates for patients with and without recurrence at the primary site were 33% and 90%, respectively ($p<0.001$).

5.6. Predictive value of histopathological parameters (Study III)

The ability of histopathologically analysed depth of infiltration, tumour grade, mode of invasion, and tumour diameter (pT-category) to predict occult metastases, local recurrences, and/or survival was examined in 73 patients diagnosed with T1/T2N0 SCC of the oral tongue.

The depth of infiltration and pT-category significantly correlated with the presence of occult cervical metastases, while only pT-category was found to predict local recurrence. The correlations between different depths of infiltration and the presence of occult metastases are demonstrated in Table 4. A specific cut-off value for depth of infiltration separating high-risk and low-risk patients was not found; the risk of occult metastasis increased gradually with increasing depth of infiltration starting from the most superficial tumours, as demonstrated in Figure 5. The rates of recurrence at the primary site for pT1 and pT2 tumours were 6% (3/49) and 25% (6/24), respectively ($p<0.05$). Neither of these variables significantly predicted survival.

The mode of invasion and tumour grade did not significantly predict occult metastases, local recurrences or survival.

A positive correlation was found between tumour diameter and depth of infiltration: depth of infiltration increases with increasing tumour diameter.

Table 4. Different cut-off points in depth of infiltration

Depth of infiltration	Pts. with occult metastasis (%)	No. of cases	p value	5-year DSS (%)	p value
≤2 mm	2 (14)	14	0.057	78	NS
>2 mm	21 (36)	58		81	
≤3 mm	2 (11)	18	< 0.05	83	NS
>3 mm	21 (39)	54		79	
≤4 mm	5 (18)	28	< 0.05	82	NS
>4 mm	18 (41)	44		79	
≤5 mm	6 (17)	35	< 0.01	82	NS
>5 mm	17 (46)	37		79	
≤6 mm	10 (23)	44	< 0.05	85	NS
>6 mm	13 (46)	28		75	

Abbreviations: DSS, disease specific survival; NS, non significant

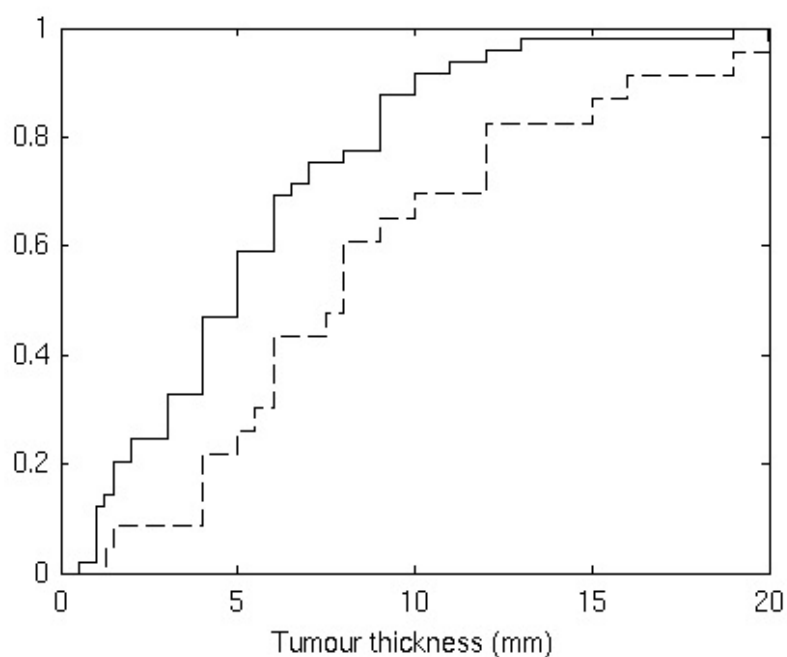


Figure 5. Empirical cumulative distribution functions of depth of infiltration for the patients without (solid line) and with occult metastases (dashed line). The steady increase of the curve of patients with occult metastases indicates that risk for occult metastases increases steadily with increasing tumour thickness. The significance of difference between the distributions was tested using the Kolmogorov-Smirnov test ($p < 0.05$).

5.7. Sentinel lymph node biopsy (Studies IV and V)

Of the 46 patients enrolled in Study IV, forty patients had END performed unilaterally and six patients with near midline tumours bilaterally resulting in 52 dissected neck-sides. The histopathological analysis revealed 10 neck sides with metastatic disease in 10 patients. In one of the patients with metastasis, a SLN could not be found. In the initial histopathological examination of the SLNs (sections at 1-2mm intervals and H&E staining), metastatic SLNs were detected in five patients. An additional histopathological examination of the initially false negative SLNs (six SLNs in four patients) was carried out including sections at 150 µm intervals and immunohistochemistry. One patient was found to have two SLNs containing micrometastasis not detected in the initial histopathological examination. These micrometastases were detected after H&E staining of additional sections. Immunohistochemical cytokeratin staining of the additional sections did not reveal previously undetected micrometastases or isolated tumor cells. In three patients, all of whom had only one SLN found at operation, the SLN did not contain metastasis although a metastasis was present in a non-SLN. The sensitivity of the method for detection of occult cervical metastases was calculated to be 67% (6/9 neck sides) and an overall accuracy of 94% (44/47 neck sides), when extensive histopathological work-up was used in cases of false negative SLN.

In Study V, initial histopathological examination with sections at 1-2 mm intervals and H&E staining revealed a metastatic SLN in two of the 13 patients (15%). No further metastases were revealed by immunohistochemical staining for cytokeratin. Both patients with a metastatic SLN had a primary tumour of the oral tongue. The metastatic SLNs were both harvested from the ipsilateral side of the neck. In one, five metastatic foci up to 3 mm were detected and in the other, a 0.4 mm micrometastasis was found. Both patients underwent a selective neck dissection (levels I-IV) and no further metastases were encountered in the histopathological analysis of the neck dissection specimens. The median follow-up time of the patients was 21 months (range, 12-42 months). All patients remained disease-free during the follow-up.

6. Discussion

Head and neck squamous cell carcinoma has a fairly high propensity to metastasise regionally via lymphatic routes. Regional metastatic spread can occur relatively early in the course of the disease. When regional metastases are present, the disease is still curable in most cases. Thus far, accurate recognition of all patients having early cervical metastatic spread has not been possible. Consequently, elective neck treatment has been offered for patients at risk resulting in a number of unnecessary neck treatments. At presentation, DM is usually encountered only in patients with locoregionally advanced disease, and when DM is present, curative treatment is usually not considered possible. For optimal treatment planning, the presence or absence of regional and distant metastases has to be evaluated. In our studies, we focused on the pre-treatment evaluation of regional and distant metastases in patients with HNSCC with special focus on patients with SCC of the oral cavity. We also analysed the benefits of the rationale to electively treat the cervical lymph nodes when the risk of occult cervical metastasis is considered high.

6.1. CT of the chest and abdomen in patients with head and neck squamous cell carcinoma

In Study I, we performed CT of the chest and abdomen on 100 patients with newly diagnosed HNSCC to clarify the indications for this examination. Only two (2%) unequivocally malignant lesions were found. Upstaging of the disease occurred in these two patients and due to the findings in the CT imaging, these patients were not treated surgically. In addition, a large aortic aneurysm was found, which had to be treated before cancer treatment. Thus, in only three patients (3%) did the CT of the chest and abdomen have an impact on the treatment of HNSCC. Two additional aneurysms were detected, one of which was operated on later. The number of DM and second primary tumours revealed by CT in our study was lower than in previous studies (Houghton et al., 1998a; Houghton et al., 1998b; Loh et al., 2005; Mercader et al., 1997; Nilssen et al., 1999; Ong et al., 1999; Reiner et al., 1997; Tan et al., 1999; de Bree et al., 2000). This can only partly be explained by the exclusion of patients with recurrent disease from our study.

Abdominal CT was negative for metastatic HNSCC or second malignancy in all 100 patients in our study. This is in concordance with previous studies, in which pathological findings from screening abdominal ultrasound have been infrequent (Houghton et al., 1998a; Nilssen et al., 1999). Therefore, abdominal imaging does not seem to be warranted in patients with previously untreated HNSCC.

Many patients had non-specific radiological findings, most often lesions in the lungs or liver. Further examinations were necessary in many cases. These further investigations did not reveal any significant findings that would have had an impact on treatment. Our results suggest that routinely performed screening with CT of chest and abdomen is not indicated in

patients with previously untreated HNSCC. Chest CT may be considered in selected cases, such as patients with very advanced stage disease.

6.2. Elective neck treatment vs. observation

The benefits of elective neck treatment were examined in Study II. In our study cohort of 80 retrospectively analysed patients with Stage I-II SCC of the oral tongue, elective treatment of the cervical lymph nodes markedly improved regional control. Significant differences in OS and DSS were not encountered. However, the patient populations in different study groups were not identical. In the ELNT group, 76% of the patients had a T2 tumour, while in the OBS group only 18% of the patients had their tumour classified as T2. We further analysed the incidence of occult metastases according to the treatment group. Occult metastases were more frequent in the ELNT group (34% versus 24%). Therefore, presuming that these two groups get equal treatment, one would expect the DSS of the OBS group to have been better. However, the 5-year DSS of the ELNT group was actually better as compared to the DSS of the OBS group (82% versus 77%) although the difference was not statistically significant. This suggests that elective treatment of the cervical lymph nodes may have had a positive effect on survival obscured by the different patient material in the study groups. The results of former studies are inconclusive. Statistically significant differences in survival between patient groups with either electively or therapeutically treated neck nodes have not been found in some studies (Duvvuri et al., 2004; Fakih et al., 1989; Vandenbrouck et al., 1980; Yii et al., 1999) while in other studies there has been a survival benefit for electively treated patients (Dias et al., 2001; Haddadin et al., 1999; Kligerman et al., 1994). Also in some other studies, regional control has been better in patients who have received elective neck treatment (Dias et al., 2001; Duvvuri et al., 2004; Spiro, Spiro and Strong, 1986).

The results of salvage treatment were unsatisfactory in our study. Only one third of all patients with a SCC of the oral tongue, who had locoregionally recurrent disease in Study II, could be salvaged. In patients, who had received elective neck treatment, the outcome of failed patients was extremely poor: only one out of nine patients with recurrent disease could be salvaged. The majority of patients in this group had already got RT, and surgery was not possible in many cases. Also in Study III, the outcome of patients with recurrence at the primary site was poor with 5-year DSS of only 33%. Comparable salvage rates in patients with locoregionally recurrent HNSCC have been reported also in other studies (Fakih et al., 1989; Kowalski, 2002; Teichgraeber and Clairmont, 1984; Yii et al., 1999).

In Study II, we found that the benefit of treating metastatic cervical lymph nodes still at the occult stage was surprisingly small. During the follow-up, 12 out of 34 patients developed cervical lymph node metastases in a previously untreated neck, out of whom 4 also had local recurrence, and seven of these patients (58%) died of their disease during the follow-up period. Fifteen patients with metastatic lymph nodes still at occult stage received elective treatment of the cervical lymph nodes. Despite treatment of the metastatic cervical lymph

nodes, seven (47%) of these patients finally died of their disease. In study III, the 5-year DSS rate for patients with occult metastases was significantly lower than for patients without occult metastases (65% vs. 91%, $p < 0.01$). These findings indicate, that despite elective neck treatment, HNSCC patients with occult cervical metastases are at great risk of death.

6.3. Predictive value of histopathological parameters

To better recognise patients at greatest risk, the value of histopathologically analysed features of the primary tongue tumour as prognostic factors was examined in Study III. Clinical and histopathological data of 73 patients with Stage I-II OTSCC were reviewed. Depth of tumour infiltration, pT-category, tumour grade, and mode of tumour invasion were re-examined and their ability to predict local recurrences, occult cervical metastases, and survival was analysed. Depth of infiltration and pT-category significantly correlated with the rate of subclinical cervical metastases. These results are in concordance with previous studies (Asakage et al., 1998; Byers et al., 1998; Fakih et al., 1989; Fukano et al., 1997; Kurokawa et al., 2002; O-charoenrat et al., 2003; Okamoto et al., 2002; Po Wing Yuen et al., 2002). Only pathological T-category significantly predicted the occurrence of local recurrence in our study. The mode of invasion classified as pushing border resulted in significantly less occult metastases than when invasion was classified as diffuse; no other significant differences were found between the proportions of patients with occult metastases in different invasion mode groups, however. This may be due to the small sample size, as only 11 patients had tumours with invasion mode classified as destructive. Some former studies suggest that mode of invasion significantly predicts the presence or absence of nodal metastases in patients with oral tumours (Odell et al., 1994; Fukano et al., 1997; Sawair et al., 2003). Well delineated tumour border is associated with low risk of metastasis, while tumour border with wide-spread cellular dissociation in small groups is associated with high risk of metastasis. Further studies with larger study cohorts are needed to clarify the real predictive value of the mode of tumour invasion. We did not find tumour grade to have any predictive value, which is confirmed by many other studies (Bryne et al., 1992; Al-Rajhi et al., 2000; O-charoenrat et al., 2003; Okamoto et al., 2002; Po Wing Yuen et al., 2002; Woolgar, 2006), though opposite findings have also been reported (Byers et al., 1998; Kurokawa et al., 2002). Contradicting many previous studies, none of the histopathological parameters examined in our study had any prognostic significance with respect to survival. In previous studies, tumour thickness or depth of infiltration (Al-Rajhi et al., 2000; Brown et al., 1989; Fakih et al., 1989; Gonzalez-Moles et al., 2002; O'Brien et al., 2003; O-charoenrat et al., 2003; Po Wing Yuen et al., 2002), tumour diameter (Brown et al., 1989; Gonzalez-Moles et al., 2002), and invasion mode (Bundgaard et al., 2002) have been found to predict survival.

We did not find any specific cut-off value for the depth of infiltration separating high risk and low risk patients. According to our results, the risk of occult neck disease seems to increase quite linearly with increasing depth of infiltration (Figure 5). There is a small risk for occult metastases even in patients with very superficial tumours. In the present study, 5.5 mm depth

of infiltration as a cut-off point had the best overall accuracy in separating patients with and without subclinical nodal disease resulting in correct classification of approximately 65 % of both patient groups with and without occult metastases (Figure 6). A sensitivity of 65% in detecting patients with occult neck disease is not satisfactory. If the cut-off point in depth of infiltration is moved downwards to increase the sensitivity, the specificity falls rapidly, as demonstrated in Figure 6. Therefore, the value of depth of infiltration of the primary tumour in clinical decision-making (elective neck treatment or not) is limited.

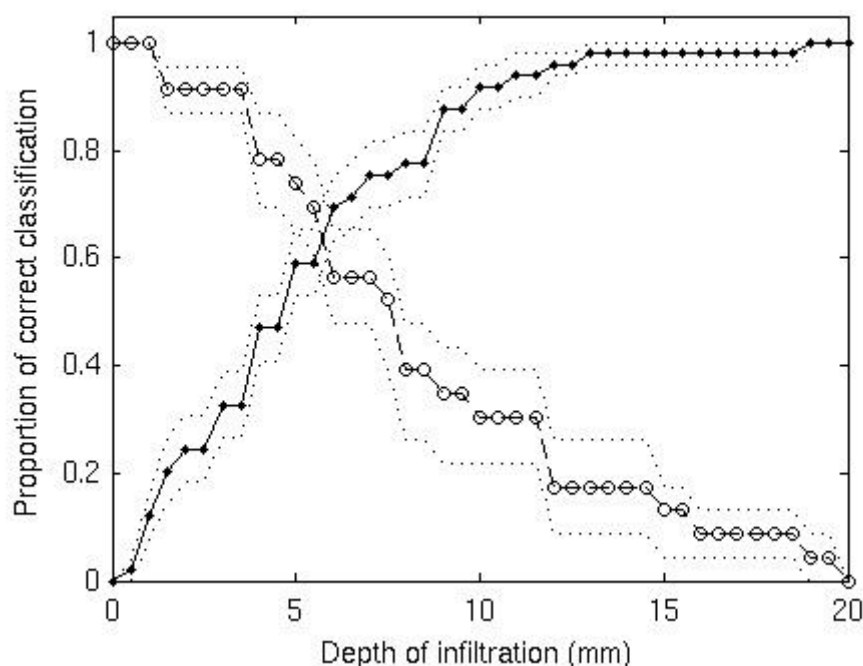


Figure 6. Proportions of correctly classified patients with either true negative necks (squares) or occult metastases (open circles) with different cut-off values of the depth of infiltration used as a decision variable to recognise patients with occult metastases. The solid line with dots indicates specificity and the dashed line with circles indicates the sensitivity. At the crossing of the curves, the overall accuracy is at its best.

6.4. Sentinel lymph node biopsy

The feasibility and accuracy of SLN biopsy in the recognition of patients with occult metastases was examined in Studies IV and V. In Study IV, SLN biopsy was performed for 46 patients who had END for T1-T3 oral cancer clinically staged as N0. Even when extensive histopathological work-up of SLNs was used, we had three out of nine patients with occult metastases in our series, who would have been incorrectly left without neck treatment had the decision been based on SLN biopsy. The sensitivity of SLN biopsy for the detection of occult cervical disease in the present study was calculated to be 67%. In many other studies published during the time we enrolled patients into our study, the sensitivity has been far

superior, exceeding 90% (Paleri et al., 2005; Ross et al., 2002; Ross et al., 2004; Stoeckli et al., 2005; Tschopp et al., 2005). Interestingly, only one SLN could be identified in all patients with a false negative SLN in our study. Hence, according to our results, it seems that the method is not reliable if only one SLN can be found. In such cases, the identification of one or more additional SLNs may have failed, or there may be an additional SLN not found because it has already been blocked by tumour tissue and therefore does not accumulate the radiotracer. Two of the patients with a false negative SLN in our series had their primary tumour in the mandibular gingiva. In these patients, the proximity of the primary tumour may have interfered with the identification of SLNs possibly located in the submandibular region.

In Study V, we performed SLN biopsy in 13 patients with a small SCC of the oral cavity (T1N0M0), who did not meet our criteria for elective neck treatment. Occult metastases were found in two of these patients, who would have been incorrectly left without neck treatment according to the current treatment protocol at our institution.

An occult metastasis may be a metastasis (>2 mm) or a micrometastasis (0.2-2.0 mm), and apart from these, isolated tumour cells (<0.2 mm) are recognised as a separate entity (Hermanek et al., 1999; Sobin and Wittekind, 2002). If one or multiple metastases are detected in SLNs, it is obvious that further treatment of the neck is indicated. The clinical significance of micrometastases and isolated tumour cells in HNSCC is not clear (Ferlito et al., 2001). In Study V we encountered a new problem: how to treat a patient with a 0.4 mm micrometastasis in a SLN? In such a case, most institutes advocate END, and in case of many positive nodes, even micrometastases, or if extracapsular spread is encountered, postoperative RT is obviously warranted. Further studies are needed to clarify the relevance of micrometastases and isolated tumour cells in clinical decision-making in the treatment of HNSCC. So far it seems that there were no false negative SLNs in this patient series, as no cervical recurrences have occurred during the follow-up with a 21 months median follow-up time.

It has been recommended that the histopathological work-up of the SLNs should include step serial sectioning of the entire SLN at 150 μ m intervals supplemented by immunohistochemistry, if metastasis is not found in conventional histopathological examination (Stoeckli et al., 2005). The examination of a single lymph node is quite laborious, when such a protocol is followed. On the other hand, only a limited number of lymph nodes, usually 1-3, need to be examined, when SLN biopsy is performed instead of END. When END is performed, the neck dissection specimen usually contains 20-40 lymph nodes, which all have to be dissected apart from the specimen and examined. In Study IV, the recommended extensive histopathological protocol was applied only on SLNs that were false negative after initial histopathological examination. Six initially false negative SLNs from three patients were additionally sectioned at 150 μ m intervals and the H&E staining of the additional sections revealed two metastatic SLNs in one patient. In Study V, immunohistochemistry was used, but the histopathological sections were made by cutting the

SLNs at 1-2mm intervals only. In our material both in Studies IV and V, the cytokeratin staining of the SLNs did not reveal further metastases or micrometastases. There are also other studies suggesting that immunohistochemistry has only a limited role in the search for metastatic SCC (Ambrosch and Brinck, 1996; Hamakawa et al., 2000; van den Brekel et al., 1992). An optimal histopathological work-up of SLNs remains to be defined. It appears that a compromise will be needed between the detection sensitivity and the histopathological workload (Thomsen, Sorensen and Krogdahl, 2005).

6.5. General discussion

A locoregional failure rate of 44% in the OBS group in Study II, consisting mainly of patients with T1N0M0 tumours, points out the need to consider more aggressive treatment strategies for patients with early tumours of the mobile tongue. Patients, who can be left without elective neck treatment, need to be selected more carefully. Evidence has accumulated, including the present study, that in SCC of the oral cavity, thickness or depth of infiltration of the primary tumour can be used to identify patients at greatest risk for having occult neck disease and who will most likely benefit from elective neck treatment. However, all patients with T1 tumours having occult metastasis can not be identified by this criteria, as demonstrated in Study III. Occult cervical disease is encountered even in patients with superficial tumours. In these patients, SLN biopsy seems to be a useful method for further assessing the clinically N0 neck.

The results of the present study have not encouraged us to replace END by SLN biopsy in patients with SCC of the oral cavity. Based on good results reported in many other studies, it seems likely that SLN biopsy will have a role in staging these tumours and that it will at least partly replace END. For most patients with oral T2 tumours, a microvascular reconstruction of the surgical defect is required. Therefore, the cervical vessels have to be uncovered for anastomosis, and END adds little to the operation time and morbidity. Elective neck dissection is also supported by the knowledge that even half of the patients with T2 tumours have been found to have occult metastases (Byers et al., 1998; Haddadin et al., 1999; Po Wing Yuen et al., 2002) and that the morbidity related to unilateral END is considered reasonable (Laverick et al., 2004). It appears that SLN biopsy will prove to be most useful in patients with T1 tumours of the oral cavity. These patients have a lower risk for occult metastasis and microvascular reconstruction of the surgical defect is usually not indicated.

7. Conclusions

1. CT of the chest and abdomen is not indicated routinely for all patients with newly diagnosed HNSCC, since it has only little impact on the treatment of these patients.
2. In patients with early SCC of the mobile tongue, a elective neck treatment results in significantly better regional control as compared to a wait and see policy, and may also have a positive effect on survival, although this could not be verified in this retrospective material.
3. Depth of infiltration and pT-category can be used to predict occult cervical metastasis, but only the pT-category predicts local recurrence in patients with early SCC of the oral tongue. Even patients with very superficial tumours may have occult metastases, and the incidence of occult metastases increases steadily with increasing depth of infiltration without any obvious cut-off value for depth of infiltration that would separate high-risk and low-risk patients.
4. Based on our present knowledge, SLN biopsy can not replace END in the treatment of oral cavity cancer, but it seems beneficial for those patients with small oral cavity tumours not planned to undergo END according to current treatment protocols.

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